NIPES

Journal of Science and Technology Research

Journal homepage: www.nipesjournals.org.ng

Assessment of Modified Azadirachta Indica Leave Extract on Some Biochemical Indicators in Rats

Okpiabhele, Augustine Osasemeaga[a *], Irabor Francis[b], Osamwonyi Sarah[c]

[a]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.
[b]Department of Biological and Chemical Sciences, Faculty of Natural and Applied Sciences, Michael and Cecilia Ibru University, Ughelli, Delta State, Nigeria.
[c]Department of Science Laboratory Technology, University of Benin, Benin City, Edo State, Nigeria.
[a]Correspondent author email:okpiabheleaustin405@gmail.com +2348101339498

ARTICLE INFORMATION

Article history:
Received 27 February 2019
Revised 07 March 2019
Accepted 13 March 2019
Available online 25 March 2019

Keywords:
Azadirachta indica
Sweetener
Medicinal
Saccharification
Astringent

ABSTRACT

Azadirachta indica is a popular tropical plant with medicinal properties used for the treatment of various infections. Thirty-five young animals were used in a 5 x 5 experimental design and were grouped according to weight proximity and labeled as Control, Test A (0.25), Test B (0.50), Test C (0.75) and Test D. (1.0). Data collected were subjected to one way ANOVA using Graph pad Prism 6.0. Serum and liver tissues were assayed for glucose, cholesterol, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT), superoxide dismutase (SOD), catalase and malondialdehyde (MDA) using standard protocols. There were significant ($p<0.05$) reductions in serum MDA, and cholesterol with a significant ($p<0.05$) increase in liver total protein in comparison with control. All other parameters were not significantly altered in comparison with control.The impact of this result is that the aspartame modified *Azadirachta indica* leave extracts could further enhance the lipid peroxidation potential of the extracts and also protect against cholesterol linked maladies.

1. Introduction

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is an artificial di-peptide sweetener, present in many unsweetened or sugar-free products [1]. The effects of aspartame have been studied on various species, including humans, rats, mice and rabbits [2]. There is a report which provides an overview of current knowledge regarding the metabolism and toxicity of aspartame. In the report, an in-depth quality assessment of 15 toxicity studies were examined, and conclusion was drawn to the effect that there was no consistent evidence that aspartame has adverse effects, either in healthy individuals or in potentially susceptible groups [3].

Phytochemicals are compounds found in plant materials. There are myriad of studies acclaiming intake of phytochemicals to reduce the risk of oxidative stress-related diseases [4, 5] and cardiovascular incidences occasioned by accumulation of cholesterol. Consumption of saturated fat increases levels of low-density lipoprotein (LDL) cholesterol associated with cardiovascular diseases (CVD). These findings have led to worldwide recommendations to reduce consumption of saturated fat to decrease the risk of CVD [6, 7].

In addition to cholesterol, lipid peroxidation products such as the lipid peroxides have been implicated as affecting health status. Lipid peroxidation is a degenerative process that affects polyunsaturated fatty acids of membrane phospholipids under conditions of oxidative stress. This process contributes to human aging and disease by disrupting the structural conformation, the packing of lipid components and ultimately, the function of biological membranes [8].

Many tropical plants which have been assayed for their antioxidant activities are used traditionally in the treatment of several human diseases. Among such plant is *Azadirachta indica* [9, 10].

Water soluble extract of *A. indica* leaves was found to possess significant hypoglycemic, hypolipidemic, hepatoprotective, anti-fertility and hypotensive activities [11].

The main discriminator against the use of neem plant extract is the bitterness associated with it, which reduces its acceptability and utilization, especially those having dislike for bitter product. This challenge has been resolved by producing sugar coated neem products [12]. The availability and affordability of these sugar coated products are issues of concern in developing regions of the world. However, whether sweeteners such as aspartame can be used to affect the taste of these extracts and still maintain the same biological effect against the aspartame free extract is of interest because such information is scarce in literature.

This study is therefore designed to investigate the effect of aspartame modified neem extract on some biochemical indicators particularly the cholesterol level and lipid peroxidation product. The importance of this study derives from the fact that the abundant tree disliked by many for its bitterness may become acceptable and hence increase the plant extract utility thereby improving the general health status particularly of the low income groups.

2. Materials and Method

2.1. Animals and Diet

A total of thirty-five male albino rats of wistar strain (120 – 150g; 4 – 6 weeks old), purchased from the animal house, College of Medicine, Ambrose Alli University, Ekpoma, were used for the study. Animals were acclimatized to laboratory conditions one week before the commencement of study, and caged in a temperature controlled room (27±1^{o}C). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines for investigations in laboratory animals.

2.2. Plant Leaves and Extracts Preparation

The leaves of *Azadirachta indica* was collected from the university premises in Ekpoma Nigeria. The plant sample was authenticated at Botany Department of the University. Leaves extract was prepared by heating 300g of fresh water washed leaves in four litres of tap water to boil at 100^{o}C for 30mins. The extract was filtered through a cheese cloth. The volume obtained was divided into five parts. To the first no aspartame was added (Control). To the second part was added 0.25g aspartame (Test A), the third part contained 0.50g aspartame (Test B), the fourth part contained 0.75g (Test C) and the fifth part 1.0g (Test D) of aspartame respectively. These extracts were kept refrigerated at 4^{o}C.

2.3. Experimental Design

Animals were allocated into five (5) experimental groups of seven rats each (n=7) as follows:

Control Group. These were given standard diet, water and aspartame free extract. Test A received standard diet, water and extract containing 0.25g aspartame. Test B received standard diet, water and extract containing 0.50g aspartame. Test C received standard diet, water and extract containing 0.75g aspartame and test D received standard diet, water and extract containing 1g aspartame.

2.4. Sample Collection

After 28 days of treatment, animals were decapitated and blood sample was collected from head wound in a lithium heparin coated tubes. A portion of blood was taken in a separate tube to harvest the serum which was stored at –20°C before analysis was carried out. Liver samples were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues were further kept in a freezer at -70^{o}C before analysis started. Serum was collected for determination of Glucose, Cholesterol, ALT, AST, Total Protein, CAT, SOD and MDA.

2.4.1. Preparation of Liver Homogenate

A portion of the liver sample was weighed, perfused with saline and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800g for 5 mins at 4^{o}C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 mins at 4^{o}C to get the post mitochondrial supernatant which was used to assay for ALT, total protein, gamma glutamyl transferase, CAT, SOD and MDA activities. To the portion to be used for MDA assay, 10 µl of Butylated Hydroxyl Toulene (BHT) (0.5M in acetonitrile) was added to prevent homogenate from oxidation and the homogenate was stored at -70^{o}C until analysis started.

2.5 Biochemical Assay

2.5.1 Estimation of Catalase Activity

Catalase activity was assayed by the method of [13].

Briefly, the assay mixture consisted of 1.96 mL phosphate buffer (0.01 M, pH 7.0), 1.0 mL hydrogen peroxide (0.2 M) and 0.04 mL PMS (10%) in a final volume of 3.0mL. About 2mL dichromate acetic acid reagent was added in 1 mL of the reaction mixture, boiled for 10 minutes, and then cooled. Changes in absorbance were recorded at 570 nm.

2.5.2 Estimation of Superoxide Dismutase

Levels of SOD in the cell-free supernatant were measured by the method of [14].

Briefly, Solution A: 1.3 mL of solution A (0.1 mM EDTA containing 50 mM $NaCO_3$, pH 10.5). Solution B: 0.5 mL 2, 3 of solution B (90 mm NBT-nitro blue tetrazolium dye). Solution C: 0.1 mL of solution C (0.6% TritonX-100 in solution A). Solution D: 0.1 mL of solution D (20 mM Hydroxylamine hydrochloride, pH 6.0) was mixed and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 mL of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) required in inhibiting the reduction rate by 50% in one minute.

2.5.3 Estimation of Malondialdehyde

As described by [15,16]; The Malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituic acid reacting substances (TBARS). Briefly, the reaction mixture consisted of 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide mLxture was brought up to 4.0 mL with distilled water and heated at 95^{o}C for 60 mins. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1 v/v) were added and centrifuged. The

organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

2.6 Assessment of Liver Enzymes and other Bio-molecules

Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) were assayed according to the method described by [17]. Gamma glutamyl transferase (GGT-Ccromatest Kit) manufactured by Linear Chemicals Spain, serum glucose [18], cholesterol [19] and total protein [20] were analyzed spectrophotometrically, using commercially prepared reagent kits from Randox.

2.7 Statistical Analysis

The results presented are the means ± Standard Errors of Mean (SEM) of three replicates. The recorded data were treated statistically using the one way analysis of variance and Instat Graph pad Prism 6.0. The means were compared by Least Significant Difference test at $p \leq 0.05$.

3. Results and Discussion

Data collected from this study shows a significant ($p<0.05$) reduction in lipid peroxidation (MDA) and catalase activities in sera of test groups administered extracts containing 0.50, 0.75 and 1g of aspartame respectively as compared with the control group. Also analysis shows that there was no significant changes ($p>0.05$) in SOD levels of various groups (Table 1). Cholesterol levels among groups administered test extracts was significantly ($p<0.05$) reduced compared with control, while other parameters remains statistically unchanged as shown in Table 2. Values obtained for liver total proteins and MDA were significantly high in comparison with the control group; all other parameters were not significantly altered in comparison with control (Table 3).

Table 1: MDA Levels and Antioxidant Enzymes Activities in Rat Serum Administered Extracts

Parameters	Control	Test A	Test B	Test C	Test D
MDA (nm/L)	2.47 ± 0.43^{a}	2.81 ± 0.71^{a}	1.32 ± 0.02^{b}	1.42 ± 0.23^{b}	1.70 ± 0.24^{b}
SOD (U/L)	1.98 ± 0.01^{a}	1.99 ± 0.01^{a}	1.98 ± 0.01^{a}	1.98 ± 0.02^{a}	2.02 ± 0.03^{a}
CAT (U/L)	25.97 ± 0.79^{a}	28.03 ± 0.90^{a}	$21.77 \pm 0.31^{c,d}$	$21.48 \pm 0.10^{b,d}$	$21.87 \pm 0.68^{d,c}$

Table 2: Some Serum Profiles in Rat Administered Extracts

Parameters	Control	Test A	Test B	Test C	Test D
Total Proteins (mg/dL)	37.50 ± 3.30^{a}	67.45 ± 2.85^{b}	72.90 ± 2.60^{b}	$57.95 \pm 0.95^{c,d}$	52.65 ± 2.25^{c}
GGT (IU/L)	3.79 ± 0.03^{a}	3.64 ± 0.12^{a}	3.79 ± 0.26^{a}	3.78 ± 0.04^{a}	3.74 ± 0.08^{a}
ALT (IU/L)	2.27 ± 0.03^{a}	$3.83 \pm 0.49^{c,b}$	3.36 ± 0.26^{b}	$2.84 \pm 0.09^{a,b,c}$	3.88 ± 0.26^{b}
MDA(nm/gTissue)	0.36 ± 0.36^{a}	0.38 ± 0.02^{b}	0.40 ± 0.03^{b}	0.41 ± 0.01^{b}	0.39 ± 0.03^{b}
SOD (U/gTissue)	1.99 ± 0.01^{a}	1.99 ± 0.01^{a}	1.99 ± 0.01^{a}	1.97 ± 0.01^{a}	1.97 ± 0.03^{a}
CAT (mm/gTissue)	39.49 ± 0.51^{a}	39.94 ± 0.02^{a}	39.98 ± 0.02^{a}	38.46 ± 1.19^{a}	39.04 ± 0.37^{a}

Table 3: Profile of Rat Liver Administered Extracts

Parameters	Control	Test A	Test B	Test C	Test D
Glucose (mg/dL)	65.26 ± 2.63[a]	65.00 ± 2.37 [a]	66.32 ± 1.06 [a]	64.47 ± 1.30 [a]	61.62 ± 0.62[a]
Cholesterol (mg/dL)	163.64 ± 2.02[a]	86.31± 3.41[e]	65.91 ± 3.99 [b,d]	73.29 ± 3.48 [d]	70.26 ± 2.37 [c,d]
Total Proteins (mg/dL)	93.01 ± 0.09 [a]	93.45 ± 0.95[a]	93.85 ± 3.56[a]	96.35 ± 0.95[a]	91.68 ± 1.62[a]
ALT (IU/L)	19.47 ± 0.68[a,b]	19.83 ± 0.87[a,b]	18.10 ± 0.86 [a]	19.14 ± 1.55[a,b]	22.45 ± 1.42[a]
AST (IU/L)	20.65 ± 0.05[a]	19.31 ± 0.79[a]	18.12 ± 0.86[a]	18.10 ± 0.86[a]	19.97 ± 0.83[a]

The process of oxidative stress produces series of highly reactive intermediates such as hydrogen peroxide, superoxide, and hydroxyl radicals which are capable of reacting with macromolecules, and lipids leading to lipid peroxidation of biological membranes [21]. As stated by [22] and [23] excessive high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death.

In this study, the concentrations of Glucose, Cholesterol, as well as the activities of ALT, AST, Total Protein, GGT, SOD, CAT and MDA were determined in the serum and liver of rats administered graded concentrations of aspartame treated with *Azadirachta indica* extract. The data shows reduction in the serum levels of cholesterol, CAT and MDA concentrations among the test groups in comparison with the control group, while all other parameters (Glucose, AST, ALT, Total Protein and SOD) were not significantly altered. The significant decrease in serum cholesterol, CAT and MDA concentrations may be attributed to the anti-hyperlipidemic and antioxidant properties of *Azadirachta indica* which represent a protective mechanism against the development of atherosclerosis, cardiovascular disease and oxidative stress. This observation was found to be consistent with the studies of [24] and [25]. [26] presented the liver as the largest gland in the human body that secretes bile and contribute to the formation of certain blood proteins and in the metabolism of carbohydrates, fats and proteins. Evaluation of liver total proteins serves as a good criterion for assessing the secretory ability/functional capacity of the liver. This study recorded a significant increase in total protein concentrations across the various groups when compared with the control group, while all other parameters were not significantly altered. Furthermore the elevation in serum total protein suggests an increase in functional activity of the liver [27].

4. Conclusion

In conclusion, *Azadirachta indica* extract adjusted with graded amounts of aspartame may be beneficial or safe as an oral remedy especially when the concentrations of cholesterol and lipid peroxidation products (e.g. MDA) are assayed for as shown in this research work. Thus extract obtained from A. Indica produces and stores in organs secondary metabolites that have been demonstrated to be active against ailments [28].

5. Acknowledgement

The authors wish to acknowledge and appreciate Metro-Research and biotechnology Africa for their technical assistance and to the editorial staff and reviewers who took part in the success of this special issue.

6. Conflict of interest

There is no conflict of interest associated with this work.

References

[1] Rowe, R. C. (2009). "Aspartame" Handbook of Pharmaceutical Excipients. pp.11 – 12 ISBN 978-158212-058-4.
[2] Mourad, I. M. (2011). Effect of Aspartame on Some Oxidative Stress Parameters in Liver and Kidney of Rats. *African Journal of Pharmacy and Pharmacology*, 5, pp. 678-682.
[3] Lesley, S. (2013). Review of Data on the Food Additive - Aspartame. Supporting Publications: EN 399. Available online: www.efsa.europa.eu/publications. pp 191.
[4] Johnson, I. T. (2004). New Approaches to the Role of Diet in the Prevention of Cancers of the Alimentary Tract**.** *Mutation Research*, 551, pp. 9 – 28.
[5] Stanner, S. A., Hughes, J., Kelly, C. N. and Buttriss, J. (2004). A Review of the Epidemiological Evidence for the Antioxidant Hypothesis. *Public Health Nutrition*, 7, pp. 407 – 422.
[6] Mensink, R. P., Zock, P. L., Kester, A. D. and Katan, M. B. (2003). Effects of Dietary Fatty Acids and Carbohydrates on the Ratio of Serum Total to HDL Cholesterol and on Serum Lipids and Apolipoproteins: A Meta-Analysis of 60 Controlled Trials. *American Journal of Clinical Nutrition*, 77, pp. 1146 –1155.
[7] Hoenselaar, R. (2012). The Discrepancy between the Scientific Literature and Dietary Advice. *Nutrition*, 28, pp.118 – 123.
[8] Robert, F. J. and Preston, R.M. (2005). Lipid Peroxidation Induces Cholesterol Domain Formation in Model Membranes. *Journal of Biological Chemistry*, 280 (47), pp. 39380 – 39387.
[9] Gayatri, N. and Sahu, R. K. (2010). Antioxidant Activity in Bark and Roots of Neem (Azadirachta Indica) and Mahaneem (Melia Azedarach). *Continental Journal of Pharmaceutical Sciences*, 4, pp. 28 – 34.
[10] Kiranmai, M., Mahender-kumar, C. B, and Ibrahim, M. D. (2011). Free Radical Scavenging Activity of Neem Tree (*Azadirachta Indica* A. Juss Var., Meliaceae) Root Bark Extract. *Asian Journal of Pharmacology and Clinical Research*, 4(4), pp. 134 – 136.
[11] Mamdouh, A. A., M., Ahmed, M. M. E., and Mona, H. (2008). Effect of Neem Leaf Extract on Freshwater Fishes and Zooplankton Community. 8th International Symposium on Tilapia in Aquaculture. 307-318. Available On: http://ag.arizona.edu/azaqua/ista/ISTA8/FinalPapers/Growth%20pdf/24.docMAMDOUH%20A.%20A.pdf
[12] Adegbite, A. E., Ayodele, M. S., Odunbaku, K.R. and Idehen, E. O. (2009). Mutagenic Effect of Neem Leaf Extract used in Traditional Medicine on Allium Cepa (L). *Scientific Research and Essays*, 4 (11), pp. 1315 – 1321.
[13] Sinha, K.A. (1972). Colorimetric Assay of Catalase. *Annals of Biochemistry*, 47, pp. 389 – 394.
[14] Kono, Y. (1978). Generation of Superoxide Radical during Auto-Oxidation of Hydroxyalamine and an Assay for Superoxide Dismutase. *Archives of Biochemistry and Biophysics*, 186, pp. 189 – 195.
[15] Shafaq, N., Nayab, R., Madiha, Q. and Tabassum, M. (2009). Reduction of Carbon Tetrachloride-Induced Rat Liver Injury by Coffee and Green Tea. *Pakistan Journal of Nutrition*, 8 (4), pp. 452 – 458.
[16] Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. *Annals of Biochemistry*, 95, pp. 351 – 358.
[17] Reitman, S. and Frankel, S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases. *American Journal of Clinical Pathology*, 28, pp. 56 – 63.
[18] Barham, D. and Trinder, P. (1972). An Improved Colour Reagent for the Determination of Blood Glucose by the Oxidase System. *Analyst*, 97(151), pp. 142 – 145.
[19] Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W. and Fu, P. C. (1974). Enzymatic Determination of Total Serum Cholesterol. *Clinical Chemistry*, 20(4), pp. 470 – 475.
[20] Doumas, B. T., Bayse, D. D., Carter, R. J., Peters, T. J. and Schaffer, R. (1981). A Candidate Reference Method for Determination of Total Protein in Serum. I. Determination and Validation. *Clinical Chemistry*, 27(10), pp. 1642 – 1650.
[21] Galano, J. M., Mas, E., Barden, A., Mori, T. A, Signorini, C., De-Felice, C., Barrett, A., Opere, C., Pinot, E., Schwedhelm, E., Benndorf, R., Roy, J., Le-Guennec, J. Y, Oger, C. and Durand, T. (2013). "Isoprostanes and neuroprostanes: Total synthesis, Biological Activity and Biomarkers of Oxidative Stress in Humans". *Prostaglandins other Lipid Mediat*ors, 107, pp. 95 – 102. doi:10.1016/j.prostaglandins.2013.04.003.
[22] Wolff, S. P. A., Garner. and Dean, R. T. (1986). Free Radicals, Lipids and Protein Degradation. *Trends in Biochemical Science*, 11, pp. 27 – 31.
[23] Jiang, Z. Y., Woollard, A. C. S. and Wolff, S. P. (1990). Hydrogen-Peroxide Production during Experimental Protein Glycation. *FEBBS Letters*, 268(1), pp. 69 – 71.
[24] Chattopadhyay, R. R. (1996). Possible Mechanism of Antihyperlipedemic Effect of Azadirachta Indica Leaf Extract, Part IV. *General Pharmacology*, pp. 958 – 960.
[25] Garima, P., Verma, K. K. and Munna, S. (2014). Evaluation of the Phytochemical, Antioxidant and Free Radical Scavenging Properties of Azadirachta Indica (Neem) Leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(2).
[26] McMinn, R. M. H. (2003). Liver and Biliary Tract. Last's Anatomy: Regional and Applied. *Elsevier*, pp. 342 – 351.
[27] Naganna, B. (2008). Plasma Proteins. In: Tawlar, G. P., Srivastava, L. M, Moudegils, K. D, Editors. Textbook of Biochemistry and Human Biology. Prentice- Hall of India Private Limited; India. pp. 172.
[28] Puri, H. S. (1991). Therapeutic Class of Neem Leaf Extracts. *Indian Medical Journal*, 10, pp. 4 – 30.